

*B1
cont
W/C1*

KCl, 60mM (NH₄)₂SO₄, 8mM MgCl₂, 1M betaine, 7mM DTE and 2mM spermidine) as described in Examples 1, 4 and 5 respectively. In another aspect of this embodiment, said amplified mRNAs are preferably capped by P¹-5'-(7-methyl)-guanosine-P³-5'-adenosine-triphosphate or P¹-5'-(7-methyl)-guanosine-P³-5'-guanosine-triphosphate in the step (e) for further in vitro translation. On the other hand, the deoxynucleotide used in the tailing reaction of said first-strand complementary DNAs is either deoxyguanylate (dG) or deoxycytidylate (dC), and the average number of tailed nucleotides is larger than seven; most preferably, the number is about twelve. Advantageously, the final amplified mRNAs can be continuously reverse-transcribed into double-stranded cDNA by Tth-like DNA polymerase activity. The final double-stranded cDNAs are preferably cloned into competent vectors for further applications, such as transfection assay, differential screening, functional detection and so on.

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W/C1*
3. In page 8, lines 6 to 20, please delete the second paragraph and replace such deleted paragraph with the following replacement paragraph:

The present invention is directed to a novel polymerase chain reaction method for mRNA amplification from single cells, named "RNA-polymerase chain reaction (RNA-PCR)". This method is primarily designed for differential screening of tissue-specific gene expressions in cell level, cloning full-length sequences of unknown gene transcripts, generating pure probes for hybridization assays, synthesizing peptides in vitro, and preparing complete cDNA libraries for gene chip technology. The purpose of the RNA-PCR relies on the repeating steps of reverse transcription, denaturation, double-stranded cDNA synthesis and in vitro transcription to bring up the population of mRNAs to two thousand folds in one cycle of above procedure. In brief, the preferred version (FIG.1) of the present invention is based on: 1) prevention of mRNA degradation (Example 1), 2) first reverse transcription and terminal transferase reaction to incorporate 3'-polynucleotide tails to the first-strand cDNAs (Example 2 or 5), 3) denaturation and then double-stranded cDNA formation based on the extension of specific promoter-primers complementary to the 3'-polynucleotide tails (Example 3 or 5), 4) transcription from

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cont* promoter to amplify mRNAs up to two thousand folds per round (Example 3 or 5), and 5) repeating aforementioned steps to achieve desired RNA amplification (Example 5).

4. Please delete the paragraph between page 8, line 21 and page 9, line 12 and replace such deleted paragraph with the following replacement paragraph:

B3
M3
M4

Alternatively, the second preferred version (FIG.2) of the present invention is based on: 1) prevention of mRNA degradation (Example 1), 2) first reverse transcription to incorporate first promoters to the 5'-ends of first-strand cDNAs and then addition of polynucleotide sequences to the 3'-ends of the first-strand cDNAs (Example 2), 3) double-stranded cDNA synthesis based on the extension of second promoter sequences complementary to the 3'-polynucleotide regions of the first-strand cDNAs (Example 3), 4) transcription to amplify either aRNAs or mRNAs up to two thousand folds in the first round of amplification cycle (Example 3), and 5) repeating aforementioned cycling steps to achieve desired amount of RNAs (Example 4). As shown in FIG.2, the first promoter used here is different from the second promoter, resulting the control of transcription by adding different RNA polymerases. The first promoter is incorporated for aRNA amplification, whereas the second promoter is designed for mRNA amplification. By this way in conjunction with a reverse transcription step, we can choose to amplify aRNAs, first-strand cDNAs, mRNAs or second-strand cDNAs of interest, depending on which RNA polymerase and nuclease we use. Although the second and third preferred embodiments (FIGS.2 and 3) are more complicated than the first preferred embodiment (FIG.1), the principle and broad features of the second and third preferred embodiments are completely within the scope of the first preferred embodiment of the present invention.

5. Please delete the last paragraph in page 9, lines 13 to 27 and replace such deleted paragraph with the following replacement paragraph:

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M4*

As used herein, the first-strand complementary DNA (cDNA) refers to a DNA sequence which is complementary to a natural messenger RNA sequence in an A-T and C-G composition. The antisense RNA (aRNA) refers to an RNA sequence which is

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W/M/T*

complementary to a natural messenger RNA sequence in an A-U and C-G composition. And, the oligo(dT)-promoter sequence refers to an RNA polymerase promoter sequence coupled with a poly-deoxythymidylate (dT) sequence in its 3'-end, of which the minimal number of linked dT is seven; most preferably, the number is about twenty-six. The sense sequence refers to a nucleotide sequence which is in the same sequence order and composition as its homolog mRNA, whereas the antisense sequence refers to a nucleotide sequence which is complementary to its respective mRNA homologue. On the other hand, the oligo(antisense polynucleotide)-promoter sequence refers to an oligonucleotide sequence which is complementary to the polynucleotide-tail of said polynucleotide-tailed cDNAs and also linked to an RNA polymerase promoter in its 5'-end. And, the Tth-like DNA polymerases refer to RNA- and DNA-dependent DNA polymerases with reverse transcription activity, such as AMV, M-MuLV, HIV-1 reverse transcriptases and C. therm. Polymerase.

6. Please delete the first paragraph in page 10, lines 1 to 16, and replace such deleted paragraph with the following replacement paragraph:

*B5
W/M/S/T*

We invent a thermal cycling amplification procedure for reproducing intracellular full-length mRNAs (Lin et. al. *Nucleic Acid Res.* 27: 4585-4589 (1999)). This cycling procedure preferably starts from reverse transcription of intracellular mRNAs with Tth-like DNA polymerases (such as reverse transcription activity of C. therm. Polymerase which is initiated with primers (SEQ ID.1, 3 or 4) at about 65~72°C for about 30~60 min as described in Examples 2, 4 and 5 respectively), following a tailing reaction with terminal transferases (at about 37°C for about 15~20 min as described in Examples 2, 4 and 5) and then denaturation of resulting mRNA-cDNA hybrid duplexes (at about 94°C for about 2~3 min as described in Examples 2, 4 and 5). After renaturation (at room temperature for 1 min or at about 52°C for about 3 min) of above tailed cDNAs to specific promoter-linked primers (SEQ ID.3 or 5), double-stranded cDNAs are formed by Tth-like DNA polymerase, such as C. therm. DNA polymerase activity at about 70°C for about 5 min (Examples 3 and 4) or Taq DNA polymerase activity at about 72°C for about 7 min (Example 5). And then, promoter-specific RNA polymerase(s), T7 or SP6 RNA